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RESEARCH ARTICLE

The determination of extract yield, polyphenolic content and anti-leishmanial activity of Iraqi *Cordia myxa* L. crude extracts obtained by two methods of extraction: A comparative study

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Abstract

Iraqi *Cordia myxa* known as Bumber, belongs to the family Boraginaceae. It contains diverse phytochemicals depending on the plant part used. Different parts of *Cordia myxa* show different phytochemicals with various pharmacological effects. The study aims to evaluate and compare the extract yields, total polyphenolic content (TPC) and total flavonoid content (TFC) of extracts obtained by optimized ultrasound-assisted extraction and conventional extraction and assess their activity against *L. tropica* promastigotes, responsible for cutaneous leishmaniasis in Iraq. Preliminary phytochemical investigation revealed that Iraqi Bumber leaf extract contains saponin, alkaloids, tannins, flavonoids, coumarins and phenolics. The optimum conditions for the highest extract yield were 15 min, 70 % and 40 kHz. The extract yield percentage obtained by ultrasound-assisted extraction was higher than that of conventional extraction methods at 14.8 % and 6.93 % of dry extract respectively. The TFC and TPC of the optimized extract were 4 and 3.5 times higher than the extract obtained by a conventional method, respectively. Moreover, the current study revealed that promastigote inhibition by Iraqi Bumber leaf extract acquired by optimized ultrasound-assisted extraction was 89 % with IC_{50} 38.98 µg/mL. In comparison, inhibition by the extract obtained by the conventional extraction method was 75 % with an IC_{50} of 46.81 µg/mL. For the first time, the TFC and TPC of the Iraqi plants were determined, along with their anti-leishmanial activities. The Iraqi plant may be a potential antileishmanial drug or be used as an adjuvant with conventional antileishmanial treatments to increase their effectiveness, decrease side effects and shorten the treatment period.

Keywords: antileishmanial activity; bumber; *Cordia myxa*; *Leishmania tropica*; polyphenols; ultrasound-assisted extraction

Introduction

Since ancient times, plants have rescued people from many illnesses. Traditional knowledge of herbal medicine has been passed down through generations in many cultures worldwide. Annually, about two-thirds of the newly identified phytochemicals are obtained from higher plants (1). Around three-quarters of the global population use plants for therapy and prevention. In America, the pharmaceutical industry is primarily based on chemical synthesis. However, approximately one-quarter of the manufactured drugs are derived from plant-based compounds (1).

Cordia myxa has many common names according to the region it is derived from. In Iraq, it is known as Bumber. The plant belongs to the Boraginaceae family and is native to the areas between the eastern Mediterranean region and eastern India (2).

The preliminary phytochemical tests conducted on *Cordia myxa* fruit extract showed the presence of oil, glycosides, flavonoids, sterols, saponins, terpenoids, alkaloids, phenolic acids, coumarins, tannins, resins, gums and mucilage (3). The polyphenolic content of leaves of *Cordia francisci*, *Cordia martinicensis*, *Cordia myxa*, *Cordia serratifolia* and *Cordia ulmifolia* was studied. The studied species showed the presence of four flavonoid glycosides (robinin, rutin, datiscoside and hesperidin), one flavonoid aglycone (dihydrorobinetin) and two phenolic derivatives (chlorogenic and caffeic acid) (4)

Traditionally, the fruits were taken orally for their demulcent characteristics to relieve cough, treat respiratory infections and relieve sore throat. The fruit's pulp was used as a paste to soften abscesses, as an emollient to relieve rheumatic pain and as an anthelminthic. Leaves macerate is used to treat trypanosomiasis and the lotion is used externally to relieve bites caused by tse-tse fly (4).The pharmacological effects of different parts and extracts of *Cordia myxa* were investigated and revealed analgesic, anti-inflammatory effects and immunomodulatory activity. Moreover, the plant showed effects on blood pressure, respiratory functions, as well as anti-stomach ulcer, antiparasitic, insecticidal, antimicrobial, antioxidant, hepato and cardio-protective effects were observed (4).

Leishmaniasis is a parasitic disease spread by the bite of infected sand flies. Two major vector species of Phlebotomus (sand fly) are involved in cutaneous leishmaniasis (CL) in Iraq, including *P. sergenti* and *P. papatasi* (5). The cutaneous leishmaniasis called, Baghdad boil, is caused by species of *Leishmania tropica*, *L. major* and *L. aethiopica*. It has been reported that *L. tropica* is responsible for the majority of CL cases (6). Cutaneous leishmaniasis is more common in rural than urban regions of Iraq. There is variability in symptoms of CL depending on the area, parasite species involved and the patient's immune response. The ulcer is painless unless a secondary infection by bacteria or fungus has developed. The sores' size may change and remain for a while. The sore may involve the nose, mouth and pharynx (7). Iraqi Centers for Disease Control (CDC) database determined the incidence of Baghdad boil cases between 2008 and 2015 in Iraq. The highest percentage (53 %) of CL cases was reported in the Iraqi middle and western regions. On the contrary, the lowest rate (1 %) of CL cases was observed in the Iraqi Northern areas (8).

Current therapies are involved in treating CL with variable efficacies and side effects. Topical application of paromomycin on lesions showed a wide range of cure rates (39 %-82 %) with a lower rate of response in infections caused by *L. tropica* (9). While the treatment with liquid nitrogen is painful and may result in scarring. Additionally, Intralesional (IL) antimonial injections, like sodium stibogluconate (SSG), are regarded as effective with an 83 % cure rate, yet are very painful and hence cause inconvenience in many patients, especially children, unless performed under anaesthesia (9). Due to these problems associated with each treatment and the discomfort or adverse effects experienced by the patients, in addition to the high incident rate of CL among Iraqi children which may result in treatment intolerance and patient non-compliance (8) . It necessitates the search for potential remedies that are effective against CL with lower side effects, toxicity, pain and burden on patients concerning the cost or admission requirement.

Newer extraction technologies, such as ultrasound-assisted extraction, possess several advantages. These include the use of low or no heat throughout the process, maintaining the stability of the extracted target compounds, eliminating the use of toxic chemicals, minimal amount of solvent utilized, shorter time of extraction, lower operational costs, enhanced process efficiency and increased yield and quality of the extracted products (10).

This study aims to evaluate and compare the extract yields and polyphenolic contents of extracts obtained by optimized ultrasound-assisted extraction and conventional extraction against *L. tropica* promastigotes responsible for cutaneous leishmaniasis in Iraq.

Materials and Methods

Plant material

The Iraqi Bambar plant was bought in February 2024 from local nurseries in Baghdad and certified by Dr. Sukaina Abbass at the College of Science, University of Baghdad. The leaves were separated from the plant and washed with tap water to remove dirt. Then leaves were dried in an aerated room for about two weeks, milled, weighed and kept in a tightly closed container until extraction.

Plant extraction

For defatting, two hundred grams of ground plant was soaked in 600 mL of petroleum ether at ambient temperature with occasional stirring for 2 days. After extract filtration, the plant material was dried to eliminate solvent remnants. The ground plant was divided into two equal parts. One part was extracted with ethanol by UAE using a probe-type ultrasonicator (Qsonicator LLC/USA), while the other part was extracted using the conventional method. The UAE experiments were conducted with different parameters, including solvent concentrations, time and ultrasonic frequency (11).

Single-factor experimental design

A single-factor experimental design was used to assess optimized conditions for the extraction of the Iraqi *Cordia myxa* leaves. The effect of three factors, each factor with three variables was studied, which included ethanol concentration used for extraction (50 %, 70 % and 90 %), the time of extraction (5, 10 and 15 min) and ultrasound frequencies used (20, 40 and 60 kHz). At the same time, the dried plant to solvent ratio (1:10) and temperature (25 °C) were kept constant in all experiments. By using a probe-type ultrasonicator, the dried plant leaves were extracted as a result of their exposure to ultrasonic waves and the obtained extracts were filtered and dried under vacuum at 40 °C in a rotary evaporator. The dried residues were stored at 4 °C in the refrigerator until further investigation. Each experiment was repeated 3 times and the mean ± SD of extract yield was calculated.

Extraction by the conventional method

For the conventional extraction, the variables used were based on the optimal conditions determined by UAE, excluding ultrasound frequency, to compare extraction efficiencies between the two methods. The plant material was macerated in 70 % ethanol in a solid-liquid ratio (1:10) for 24 hr with occasional stirring. The extract was filtered and the filtrate was dried by a rotary evaporator, weighed and stored in the refrigerator until use. The plant residue was re-extracted several times until a clear filtrate appeared.

Preliminary phytochemical evaluation of crude ethanolic leaf extracts

Several chemical tests were employed in phytochemical investigations of crude extracts obtained by ultrasound-assisted and conventional extraction methods (12).

Saponin (Foam test)

In a test tube, 1 mL of extract was added to 9 mL of distilled water, agitated for 15 s and left to stand for 15 min. Persistent foam of 1-2 cm height was considered positive.

Alkaloid (Dragendroff's test)

Dragendroff's reagent was added to a small portion of the extract. The appearance of orange-brown insoluble residue indicated the existence of alkaloids.

Terpenoids (Salkowski test)

A small portion of the extract was dissolved in 2 mL of chloroform and then added 3 mL of concentrated H_2SO_4. The appearance of a reddish-brown colour indicated terpenoids.

Sterols and steroids (Libermann-Burchard test)

A portion of the plant extract was dissolved in 5 mL of chloroform, then anhydrous sodium sulfate was added to a dry chloroform layer, after which acetic anhydride (10 drops) and concentrated sulfuric acid (2 drops) were added. The appearance of a bluish-green colour indicated a steroidal nucleus.

Tannins (10 % lead acetate)

Mixing 1 mL of the extracts with 1 mL of lead acetate (10 %) solution indicated the presence of tannins by the appearance of a white precipitate.

Flavonoids (Alkaline reagent test)

A few drops of sodium hydroxide solution were mixed with 1 mL of extract. The appearance of a yellow colour indicated the flavonoids.

Coumarins (Fluorescence response test)

A few drops of extract were added to the silica TLC plate, dried and then sprayed with 1 % reagent of KOH. Blue-green fluorescence indicated the presence of coumarins.

Polyphenols (5 % ferric chloride)

1 mL of extract and 1 mL of 5 % ferric chloride solution were added. Deep green or deep blue indicated the presence of polyphenolic compounds.

Total flavonoid content (TFC) estimation

An aluminium chloride assay was implemented to estimate the TFC of the extracts of each experiment. Ethanolic Rutin standard solution was prepared and then serial dilutions of 1, 0.50, 0.25, 0.125 and 0.065 mg/mL were prepared. In a test tube containing 1 mL of the standard, 0.3 mL of 5 % $NaNO_2$ was added and left for 5 min. Then, 0.3 mL of 10 % $AlCl_3$ was added to the solution and left for 5 min. After that, 2 mL of 1M NaOH solution was added to the solution in the test tube, followed by distilled water to make up the volume to 10 mL. Finally, the solution was incubated for half an hour at ambient temperature. A UV spectrophotometer was used to determine the absorbance of the solution at 510 nm. The solutions of extracts (1 mg/mL) and blank were prepared using the same procedure. The Rutin standard curve was established by graphing each concentration versus its corresponding absorption. The TFC was denoted as (mg RE/g) of dried plant. The estimation of TFC was performed in triplicate (13).

Total phenolic content (TPC) estimation

The Folin-Ciocalteu method was used to determine the total phenolic content of the extracts from each experiment. Aqueous gallic acid standard solution was prepared and the serial dilutions with concentrations of 1, 0.5, 0.25, 0.125, 0.0625 and 0.0312 mg/mL were prepared. In a test tube, 1 mL of the standard was added. Then, 5 mL of distilled water and 0.5 mL of Folin-Ciocalteu's reagent were added to the test tubes. The solution was left for 5 min, then 1.5 mL of 20 % Na_2CO_3 was added and the volume was made up to 10 mL with distilled water. The solution was left for 90 min at ambient temperature. A UV spectrophotometer was applied to determine the absorbance of the solution at 760 nm. The extracts (1 mg/mL) and the blank solutions were prepared using the same procedure. Each concentration of the gallic acid standard was plotted against its corresponding absorption to establish the gallic acid standard curve. The TPC was denoted as (mg GAE/g) of dried plant. The test was repeated three times (14).

Test for anti-leishmanial activity

The anti-leishmanial activity was conducted according to the previous protocol (15) with some modifications. One mg of each extract from each extraction method was diluted by 0.5 % (v/v) dimethyl sulfoxide (DMSO) to acquire a concentration of (1 mg/mL) from which serial dilutions were prepared (1000, 500, 250, 125, 62.5, 31.25, 15.6, 7.81 and 3.9 µg/mL). One mL of sodium stibogluconate injection (100 mg/mL) was diluted with distilled water to a final volume of 100 mL to acquire a concentration of 1000 µg/mL. Serial dilutions were also prepared (1000, 500, 250, 125, 62.5, 31.25, 15.6, 7.81 and 3.9 µg/mL). The antileishmanial drug was used as a positive control, while DMSO was a negative control. All test solutions were filtered through 0.22 µm Millipore membranes under aseptic conditions before seeding. The promastigotes of *Leishmania tropica* were obtained from the Biotechnology Research Center / Al-Nahrain University and were employed to assess the antileishmanial activity of plant extracts. The promastigotes were isolated from patients with cutaneous leishmaniasis (CL) in the southern parts of Iraq, where CL is endemic. The late log-phase promastigotes were incubated in a 12 % fetal calf serum-enriched RPMI medium. For the assays, 100 µL of promastigote culture (10^6 cells/mL) was seeded in each well of 96-well flat-bottom plates. Then 10 µL of each test extract concentration was added to wells. Plates were then incubated for 24 hr at 25 ± 1 °C. Then, 10 µL of MTT reagent was incorporated into each well and the plates were incubated for 4 hr at 25 ± 1 °C to assess cell metabolic activity. After which, DMSO was added and incubated for half an hour as a solubilizing solution. A multi-well scanning spectrophotometer (ELISA reader) (Organon Teknika microwell system reader 230 s/Belgium) was used to measure the relative optical density (OD) at 490 nm. The number of viable cells directly correlated with the absorbance of the formazan produced by metabolically active cells. The assays were performed in triplicate. The inhibition percentage was calculated according to the formula given. The results were expressed as mean ± SD (15). Sample OD is the absorbance of the test sample.

$$\text{Percentage of inhibition} = \frac{\text{Control OD - Sample OD}}{\text{Control OD}} \times 100 \quad \text{(Eq. 1)}$$

Analytical statistics

All experiments were carried out in triplicate and demonstrated as mean ± standard deviation. Calibration curves were constructed using Excel 2016. One-way ANOVA was employed to determine the influence of various factors

on extraction efficiency. Non-linear curve fitting was implemented to calculate IC_{50} values. A p-value ≤ 0.05 was regarded as statistically significant. GraphPad Prism 8 was used for these calculations.

Results and Discussion

The efficiency of both extraction methods was compared by evaluating extract yields, flavonoid content and phenolic content of Iraqi *Cordia myxa* obtained by optimized conditions of UAE and by the conventional extraction method based on several parameters used in UAE.

Analysis of a single-factor experiment of UAE

In the current study, the parameters of UAE were optimized using a single-factor experimental analysis.

Determination of the effect of extraction time on extract yield

One of the critical parameters affecting extraction efficiency is time. The optimal time for the UAE was evaluated for each plant and its studied part. To assess the effect of this variable, the experiments were carried out for 10, 15 and 20 min with other parameters held constant such as 70 % ethanol, 40 kHz frequency and solvent/solid ratio (10:1). The optimized time was 15 min for the maximum extract yield (1.48 ± 0.02 g/10 g) (14.8 %), but further increase in time to 20 min decreased the extract yield to (0.87 ± 0.01g/10 g) of dry plant as demonstrated in (Table 1 & Fig. 1). The time factor significantly influenced (p ≤ 0.0001) the extraction efficiency. A previous study showed that 15 min was the optimal extraction time of another species of Cordia, in particular *Cordia dichotoma,* with an extract yield of 19.53 % which is in agreement with the current study in terms of time factor but the yield is higher than Iraqi Bumber may be due to interspecies differences (16). The current study's also agreed with earlier studies which showed that extending the extraction time from 5 to 15 min increased the extraction yield of phenolics from *Ziziphus jujuba* (17). A positive linear effect of time was observed on the yield and phenolic compounds obtained from the pomace of grapes by UAE total flavonoids from grape pomace and hawthorn seed (18). While an adverse linear effect of time was demonstrated on the yield of pectin derived from sour orange peel and phenolic compounds from the shell powder of coconut (*Cocos nucifera*) in the range of 20–60 min (19). As noticed, the time factor was influenced by the type of phytochemical to be extracted and the plant part. Initially, the yield increased when the extraction time increased and decreased when the time was extended (20). An increase in the initial time of extraction enhanced the solvent penetration into the matrix of plant tissue due to the effect of cavitation of ultrasound, thus increasing the contact between the solute from the plant and the extraction solvent and assisting their diffusion into the solvent. The prolonged exposure of plant material to ultrasound resulted in solute damage and decreased extraction yield (20).

Table 1. Experimental conditions of UAE and extract yield of Iraqi *Cordia myxa*

Exp.	Time(min)	Solvent (%)	Freq.(kHz)	Yield g/10 gm
1	10	70	40	0.976 ± 0.005
2	15	70	40	1.486 ± 0.025
3	20	70	40	0.873 ± 0.015
4	15	70	20	0.733 ± 0.011
5	15	70	60	0.816 ± 0.0164
6	15	50	40	0.94 ± 0.01
7	15	90	40	0.71 ± 0.017

Determination of the effect of the concentration of solvent on extract yield

In this study, phytochemicals were extracted from the Iraqi plant using different ethanol concentrations. Ethanol was chosen because it is affordable, environment-friendly, categorized as a safe solvent and has a high affinity for most phytochemicals, in particular for phenolics. To examine how hydro-alcohol concentrations affect yield extract, various concentrations of water/ethanol solutions were employed while other parameters remained constant. Table 1 and Fig. 2 illustrate the impact of solvent concentrations on extraction efficiency. The solvent concentration effect was highly significant (p ≤ 0.0001) about extract yield. The highest extract yield was observed with 70 % ethanol and determined as the optimal concentration for efficient extraction. The optimal solvent concentration determined in the present study agreed with previous research which revealed that an increase in alcohol concentration to 70 % led to an increase in phenolic and flavonoid contents (21). In contrast, another study reported that the optimal ethanol concentration for UAE was 50 % in combination with other

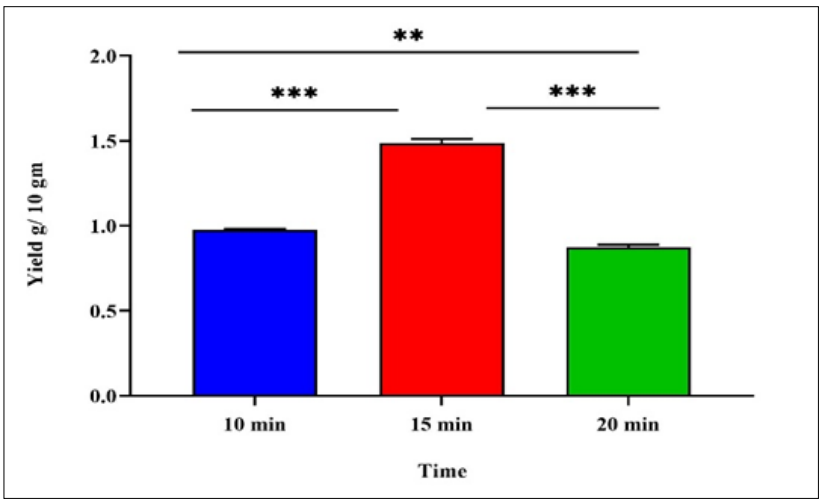

Fig. 1. Effect of extraction time on the Iraqi *C. myxa* leaf extracts yield.

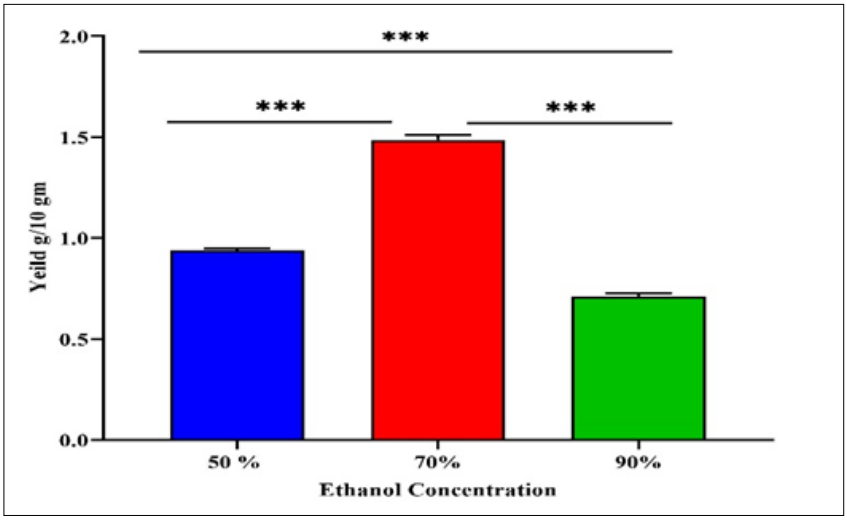

Fig. 2. Effect of extraction solvent concentrations on the Iraqi *C. myxa* leaf extracts yield.

optimal conditions, leading to considerable contents of phenolics and flavonoids, as well as the antioxidant effect from mango residue (22). In the current study, the yield declined to 0.71 ± 0.0172 g when the ethanol concentration was elevated to 90 %. The results agreed with other investigations that observed the same behaviour in flavonoids' extraction from solid wastes of grapefruit (23). This may have resulted from a decreased solvent's dielectric constant when the concentration of alcohol increased, which contributed to an increase in solubility and diffusion of the phenolic compounds. The yield of phenolic compounds was increased when ethanol concentration increased and an adverse effect on the yield was observed when the maximum alcohol concentration was reached. Absolute ethanol (near 100 %) may result in dehydration of plant tissue, protein denaturation and lower yield. A study disagreed with the current results and showed that an elevation in alcohol concentration increases phenolic compounds' yield derived from grape seeds and further increases the highest alcohol concentration at stable temperature and extraction time (18).

Determination of the effect of ultrasound frequencies on extract yield

The ultrasound frequency may be a pivotal factor that influences the extraction effectiveness and is missing in conventional methods. This experiment showed that an increase in the ultrasound frequency from 20 kHz to 40 kHz elevated the yield from 0.733 ± 0.011 g/ 10 g to 1.486 ± 0.025 g/10 g, proving an increase in extraction performance. An additional increase in the frequency to 60 kHz reduced the yield to 0.816 ± 0.0164 g (Table 1 & Fig. 3). These observations agreed with a previous study that showed a decline in the extract yield of *Lepidium sativum* when ultrasonic frequency increased (11) . Researchers studied the influence of varying frequencies (40, 80 and 120 kHz) on phenolics extraction obtained from pomace of grapes, which showed that 40 kHz was most effective, which was in agreement with the optimal ultrasonic frequency of the current study (24) . Also, the current study's findings agreed with another researcher who demonstrated that an increment in ultrasonic frequency from 18 to 54 kHz elevated the anthocyanin yield from strawberries (25) .

Cavitation bubbles form and grow when there is a definite time of compression and rarefaction cycle, but these bubbles will fail to form and grow if the cycle is very short. There is an opposite relation between the time of the rarefaction phase and ultrasound frequency; so at higher frequencies, the cavitation bubble grows in a very short duration and prevents its collapse. The enormous bubbles created at elevated frequencies provided additional opposition

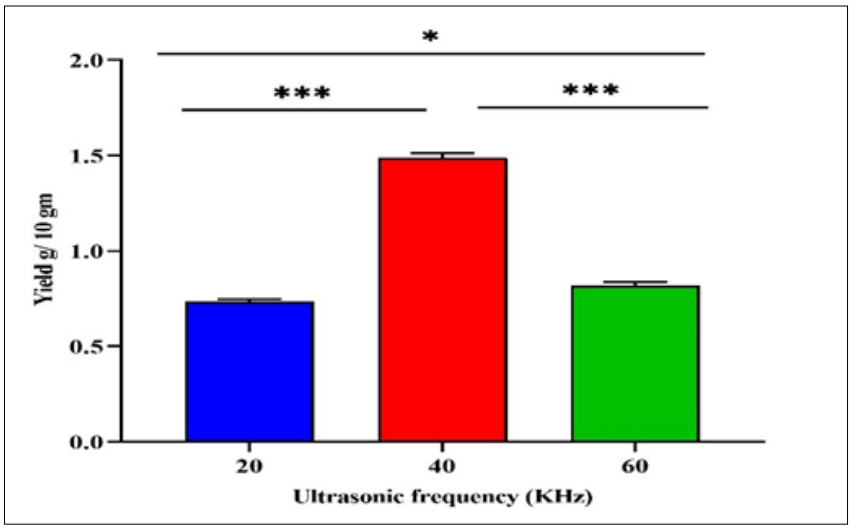

Fig. 3. Effect of ultrasound frequency on the yield of the Iraqi *C. myxa* leaf extract.

to mass transfer (26). As observed from the studies, as mentioned earlier, optimization of extraction frequency should be determined for each plant species, matrix and phytochemical to be extracted. The optimal frequency for the highest extract yield of the Iraqi plant was 40 kHz.

Conventional method for extracting yield

The extract yield obtained by maceration was 0.693 g/10 g (6.93 %) of the dry plant. As observed, the yield obtained from UAE was about 2 times greater than the yield obtained from the conventional method (Fig. 4). The yield obtained by maceration was higher than some previous studies (27–29) obtained leaf extract yield of 4.65 % in Indonesia, 6.8 % in India and 0.284 g in Iraq. The extract yield of the current study was lower than 7.14 % for Indonesian *Cordia myxa* (30). A study revealed that extraction yields obtained by UAE and Soxhlet extraction of *Cordia dichotoma*, a different Cordia species, were 19.53 % and 17.01 %, respectively (16). While *Cordia glabrata* showed an extract yield of 1.362 % obtained by maceration in absolute ethanol (31). It has been noticed that the variation in extract yield among different studies may be influenced by the extraction method, extraction temperature, extraction solvent, concentration of extraction solvent, plant material to solvent ratio, species of plant, origin of plant and part used.

Preliminary phytochemical determination of the Iraqi *Cordia myxa* leaf crude extracts

The hydroalcoholic extracts acquired from the two extraction methods were tested by easy, fast and cost-effective phytochemical screening assays (Table 2). The preliminary phytochemical analysis of the extract obtained by UAE revealed more intense colours by alkaloids, Tannins, Flavonoids and Phenolics tests than the extract obtained by CE, indicating a higher concentration of phytochemicals. A qualitative investigation of the petroleum ether fraction was positive for terpenoids and steroids, while negative for hydroalcoholic extract. Some preliminary analysis results were consistent with another research conducted on *Cordia myxa* leaves in Iraq, except for terpenoids (32). However, it is inconsistent with an Iraqi study that showed *Cordia myxa* extract was positive only for alkaloids and negative for other phytochemicals (33). Moreover, Iraqi fruit alcoholic extract phytochemical screening revealed the presence of many phytochemicals: carbohydrates, proteins, glycosides, alkaloids, flavonoids, phenolic compounds and tannins without saponins (34). Whereas, Pakistani *Cordia myxa* leaf methanolic/chloroform extract showed the presence of tannins, coumarins and the absence of other phytochemicals (35). The variability in the concentrations and types of secondary metabolites in fruits and vegetables mainly relies on the biosynthetic pathway through plant growth and development. Factors affecting phytochemicals in plants include cultivar variation (interspecies), environmental conditions (sun rays, temperature fluctuation and weather conditions in particular geographical areas), agronomic conditions (planting date, fertilisation, watering and subsequent plant collection that influence the yield, phytochemical content and constituents of both fruit and vegetables (36).

Determination of TFC and TPC

TFC and TPC were evaluated for the extraction efficiency and the' value of extracts acquired by UAE and CE methods. Determination of TFC of the two methods of extraction was according to the linearity of the Rutin calibration curve ($y = 1.0459x + 0.0318$) ($R^2 = 0.9964$) (Fig. 5). While, the TPC of both methods were computed according to the linearity of the gallic acid calibration curve ($y = 0.7453x + 0.2238$) ($R^2 = 0.9849$) (Fig. 6). The levels of TFC and TPC of crude leaf extracts of Iraqi *Cordia myxa* obtained by optimized conditions of UAE were higher than those obtained by CE suggesting high-quality extract (Table 3). The high extract quality of the Iraqi plant obtained by UAE may be influenced mainly by the ultrasonic frequency in addition to other

Table 2. The qualitative analysis of hydroalcoholic and petroleum ether crude extracts obtained by two methods of extraction of Iraqi *Cordia myxa* leaves

Phytochemical	UAE[a] Hydroalcohol	Maceration Hydroalcohol	Maceration Petroleum Ether
Saponin	+	trace	-
Alkaloids	++	+	-
Terpenoids	-	-	+
Sterols and steroids	-	-	+
Tannins	++	+	-
Flavonoids	++	+	-
Coumarins	+	trace	-
Phenolics	++	+	-

[a]Ultrasound Assisted Extraction

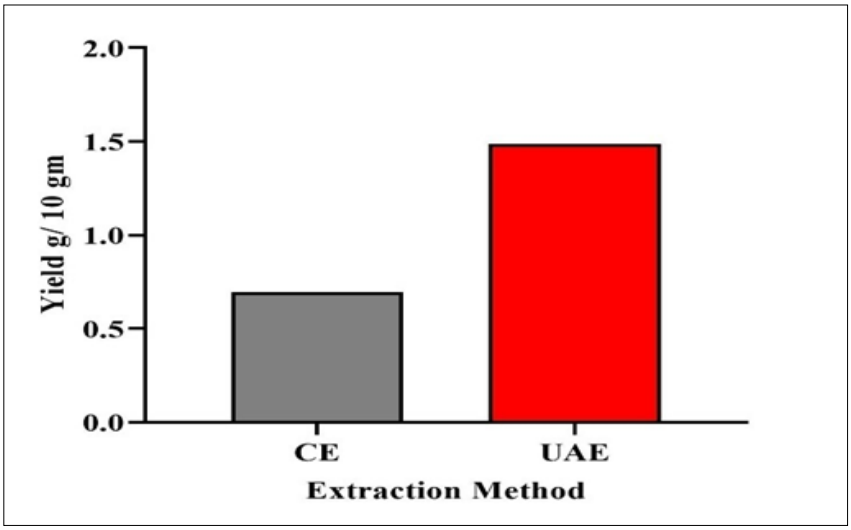

Fig. 4. The extract yields of Iraqi *C. myxa* obtained by Ultrasound-assisted (UAE) and conventional extractions (CE).

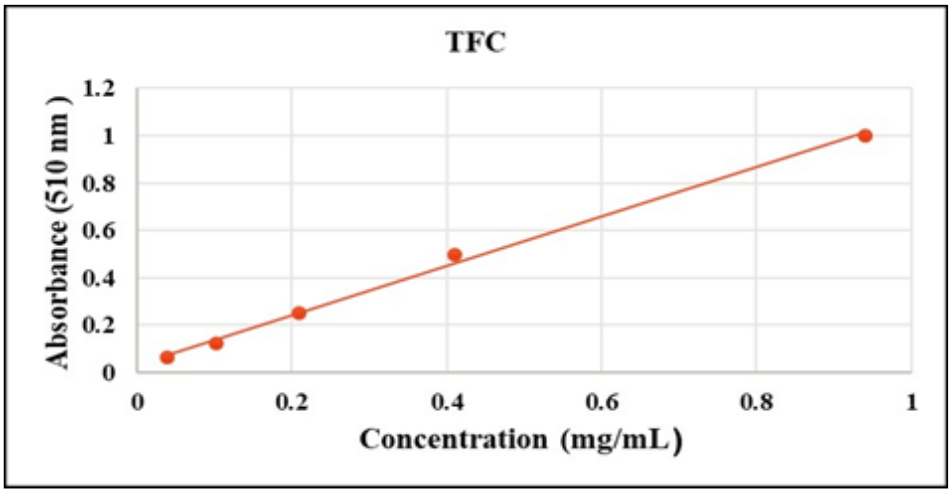

Fig. 5. Rutin standard calibration curve to evaluate TFC of Iraqi *C. myxa* leaf extracts.

Table 3. TFC and TPC of Iraqi *Cordia myxa* leaf extracts obtained by Ultrasound-Assisted Extraction (UAE) and Conventional Extraction (CE)

Total Content	TFC (mg/g) of dried plant	TPC (mg/g) of dried plant
UAE	8.26 ± 0.115	27.43 ± 0.150
CE	2.33 ± 0.097	8.94 ± 0.167

Table 4. Log (IC_{50}), IC_{50}, Inhibition (%) and R^2 of Sodium Stibogluconate (Control) and extracts obtained by UAE and CE

Log IC_{50}	**Control** 1.567	**UAE** 1.591	**CE** 1.67
IC_{50}(µg/ml)	36.87	38.98	46.81
Inhibition (%)	96.46 ± 2.0	89.26 ± 0.96	74.56 ± 0.97
R^2	0.9981	0.9929	0.9990

optimized parameters, while the CE method was conducted with similar experimental parameters of UAE, including solvent concentration, time and temperature, excluding the frequency factor.

The literature search did not show previous studies concerning the TFC and TPC of Iraqi *Cordia myxa* leaf extract. The current study showed that TPC obtained by UAE was about triple the amount obtained by the conventional method. Similar findings were demonstrated for the TFC obtained by UAE compared to CE (Table 4). The TPC and TFC of the CE were higher than those in a study, which revealed 5.25 mg/g and 0.356 mg/g, respectively, for Indian *Cordia myxa* leaves obtained by the maceration method (28). Moreover, the current study revealed that optimized UAE (15 min, 70 %, 40 kHz) contributed to higher phenolic content than CE of Iraqi *Cordia myxa* leaf extract than a study that showed that fruit's peel extract with phenolic content (11.1 ± 1.47 mg/g gallic acid equivalent) obtained by maceration (37, 38). Another study showed that the TPC of Iranian *Cordia myxa* methanolic fruit extract obtained by sonication was 0.402 mg/g, lower than the TPC of leaves of the studied plant obtained by the two methods (38). On the contrary, fruit extract of Iranian *Cordia myxa* obtained by magnetic stirrer showed higher TPC and TFC (113.71 ± 0.04 mg of gallic acid/g of dried extract and 68.9 ± 0.002 mg of quercetin/g of dried extract, respectively) (39). The comparison between the phenolic and flavonoid contents of adult and young leaves of *Cordia glabrata* showed concentrations of 89.11 ± 0.46 mg EAG g^{-1} and 15.37 ± 0.11 mg EQ g^{-1} respectively (31). It has been observed that the polyphenolic content varied according to the region, plant part, species, growth stage and method of extraction.

Evaluation of antileishmanial activity

The MTT assay is a widely used colourimetric method to assess the extent of cytotoxicity of different drugs (40). *Leishmania tropica* promastigotes were used to evaluate the potential of leishmanial inhibitory activity of the obtained extracts. Literature search did not show studies that

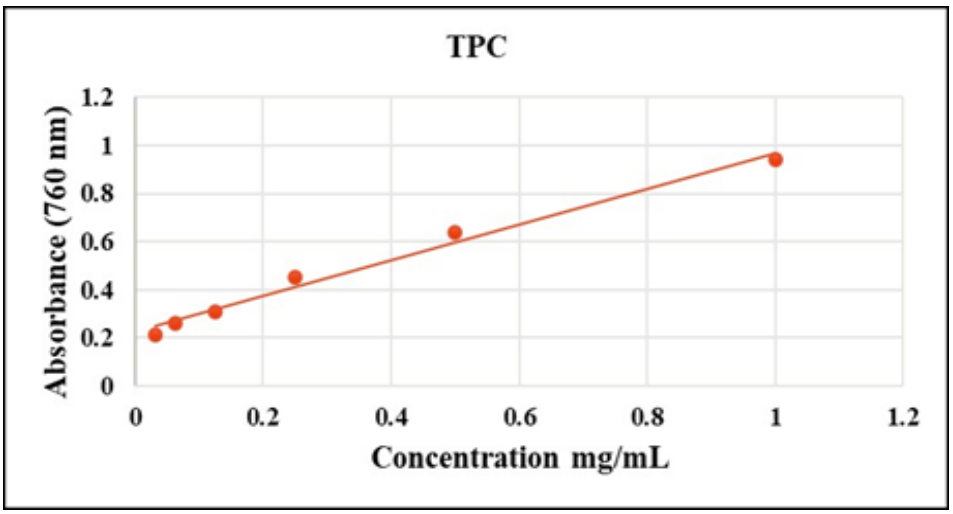

Fig. 6. Gallic acid standard calibration curve to evaluate TPC of Iraqi *C. myxa* leaf extracts.

investigated the leaf extract of *Cordia myxa* against *L. tropica* promastigotes. The antileishmanial activity was dose-dependent and expressed as a percentage of inhibition (%) and the IC_{50} (µg/mL) was determined by plotting inhibition (%) against Log (concentration) of each extract and control (Table 4 & Fig. 7). The current study revealed that promastigotes inhibited by Iraqi Bumber leaf extract acquired by optimized UAE was 89.26 ± 0.96 % with IC_{50} 38.98 µg/mL. In contrast, the inhibition by the extract obtained by CE was 74.56 ± 0.97 % with IC_{50} 46.81 µg/mL. Whereas, the control showed an inhibition of 96.46 ± 2.0 % with a half-maximal concentration of 36.87 µg/mL. The IC_{50} of the optimized UAE extract was close to the IC_{50} of the positive control, indicating an extract of high potency and high quality with higher polyphenolic compounds than the extract obtained by CE.

It was earlier noted that *C. myxa* mucilage extract was effective on two promastigote species of Leishmania, including *L. major* and *L. infantum*, with an IC_{50} of 26 ± 2.2 mg/mL and 35 ± 2.2 mg/mL, respectively (41). The percent inhibition after 72 hr treatment of *L. major* and *L. infantum* promastigotes was 16.68 % and 16.68 %, respectively, at the extract's highest concentration. The study showed a lower inhibition percentage and higher half-maximal concentration compared to the present study, suggesting that Iraqi Bumberl leaf extracts are richer in phytochemicals than the mucilage extract of the plant and *L. tropica* promastigotes are probably more sensitive to the extracts than *L. major* and *L. infantum*. Many studies of different plant extracts have been conducted against other species of cutaneous leishmaniasis. Species-specific variability in drug sensitivity necessitates individual testing of each extract. It has been observed that there was species variability in sensitivity toward polyphenolic extracts of different plants. It was observed that there are differences between species and unpredictable behaviour toward drugs. So, each drug must be investigated on a species-by-species basis to guarantee its effectiveness (42). Phytochemical investigations considered that Bumber is rich in trace elements (such as selenium, copper, zinc, iron and manganese), phenolic and flavonoid compounds (robinin, datiscoside, rutin, hesperidin, dihydrorobinetin, caffeic acid and chlorogenic acid) (43). Reports are available on the impact of selenium compounds on leishmanial activity. The investigation mentioned that selenium-containing compounds, considered as the new anti-leishmanial drugs against two forms of Leishmania parasite (amastigotes and promastigotes), possess more powerful activity and reduced cytotoxicity than conventional treatments (44). Several studies concerning flavonoids' antileishmanial activity have been conducted. The biological processes of the leishmania parasite depend on Iron for growth and survival. The chelating property of flavonoids may be exploited to chelate the iron of the parasite and inhibit growth by causing mitochondrial dysfunction and changing the iron-dependent enzyme expression (45). The ability of flavonoids to chelate iron may contribute to alterations in cell morphology and cycle (45). The metal complexes of Fe (II), Fe (III) and Cu (II) ions with rutin, taxifolin, epicatechin and luteolin showed significantly greater effect against leishmania than free flavonoids (46). Bumber plant contains flavone derivatives and Rutin and the literature has revealed the impact of flavones against leishmania through different mechanisms (4). The antileishmanial activity of *Arrabidaea chica* against promastigotes of *L. amazonensis* when exposed to flavone-rich fraction and Luteolin resulted in subcellular changes of the parasite, such as extreme vacuole formation in cytoplasm and swelling of mitochondria (47). The effects of Rutin against sodium stibogluconate (SSG) sensitive (S-) and resistant (R-) strains of *L. donovani* are evaluated (48). The study demonstrated that the anti-promastigote effect of Rutin was through cell arrest at the G0/G1 phase. NF-κB and iNOS gene expression were up-regulated by Rutin in mice infected with sensitive and resistant strains. Also, it has been shown that flavonoids could combat anticancer and antileishmanial drug resistance by binding to the nucleotide-binding domains (NBD) of the ATP-binding cassette (ABC) transporters. These cellular episodes increase in hydrophobic interactions, leading to multi-drug resistance inhibition (49).

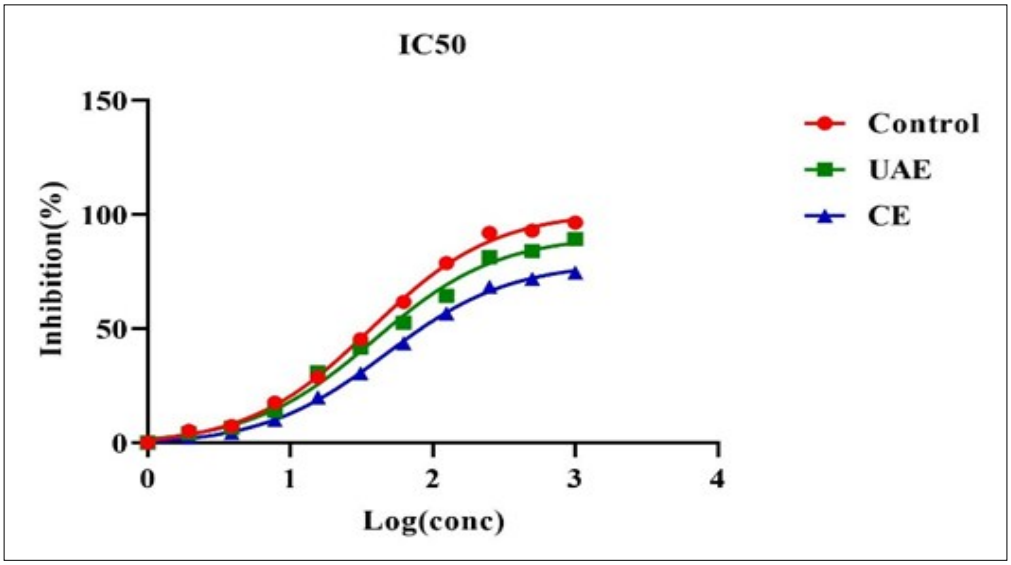

Fig. 7. Percentage of inhibition of *L. tropica* promastigotes by control and Iraqi *C. myxa* extracts obtained by optimized UAE and CE against Log (concentration) values.

Conclusion

Using two extraction techniques, this is the first to investigate the polyphenolic content and antileishmanial activity of Cordia myxa leaves from Iraq. UAE obtained the highest yield of extract with optimal conditions of 15 min, 70 % ethanol and 40 kHz at constant temperature and solvent-to-solid ratio. The TFC and TPC results of the optimized UAE were approximately 4 and 3 times higher than CE, respectively. The efficiency of the UAE exceeds conventional extraction by improving the yield and lowering solvent consumption with a shorter extraction time. The inhibitory effect of *L. tropica* promastigotes was greater with the optimized extract than with the extract by a conventional method, indicated by the lower IC_{50}. The high potency of the optimized extract may be due to its high quality and higher polyphenolic content compared to the extract obtained by CE. The Iraqi plant may be a potential antileishmanial drug or be used as an adjuvant with conventional antileishmanial treatments to increase their effectiveness, decrease side effects and shorten the treatment period. Further, *in vivo* studies are important to assess the antileishmanial effect of Iraqi *Cordia myxa* leaf extract.

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Compliance with ethical standards

Conflict of interest: The Author has no conflict of interest to declare.

Ethical issues: None

References

1. Orhan IE. Biotechnological production of plant secondary metabolites. Bentham ebook. 2012:107. https://doi.org/10.2174/97816080511441120101
2. USDA, ARS, National Genetic Resources Program. Germplasm Resources Information Network (GRIN). Beltsville (MD): National Germplasm Resources Laboratory; 2015 [cited 2024 Jul 6]. Available from: https://npgsweb.ars-grin.gov/
3. Rashed K, Luo MT, Zhang LT, Zheng YT. Evaluation of anti-HIV-1 activity of *Cordia myxa* L. and phytochemical profile. Banats J Biotechnol. 2015;5(10):75–82. https://doi.org/10.7904/2068-4738-V(9)-51
4. Al-Snafi AE. The Pharmacological and Therapeutic Importance of *Cordia myxa*- A review. IOSR J Pharm. 2016;6(6):47–57.
5. Al-Obaidi MJ L, Ibrahim K, Adnan T, Abd Al Hadi E, Akram E, Qazi Z, et al. Epidemiological study to investigate a possible vector of visceral leishmaniasis in the central region of Iraq. Al-Mustansiriyah J Sci. 2013;26(6):1–12.
6. Aljeboori, TI, Evans D. 1980. *Leishmania* spp. in Iraq. Electrophoretic isoenzyme patterns. II. Cutaneous leishmaniasis. Trans R Soc Trop Med Hyg. 1980;74(2):178–84. https://doi.org/10.1016/0035-9203(80)90239-4
7. Brunette GW, Kozarsky PE, Cohen NJ, Gershman MD, Magill AJ, Ostroff SM, et.al. Communicable disease control centre. Health information for international travel: the yellow book. New York, NY: Oxford University Press; 2016. p. 688. https://doi.org/10.4269/ajtmh.16-0627
8. Al-Obaidi MJ, Abd Al-Hussein MY, Ihsan M. Al-Saqur, Survey study on the prevalence of cutaneous leishmaniasis in Iraq. IJS. 2016;57 (3C):2181–87.
9. Yuval YR, Enk CD, Murad S, Yofe V, Gozal D, Pessach VM. Intralesional sodium stibogluconate under inhaled anaesthesia for the treatment of cutaneous leishmaniasis in children: A retrospective cohort. J Am Acad Dermatol. 2019;81(4):1013–15. https://doi.org/10.1016/j.jaad.2019.01.083
10. Tiwari BK. Ultrasound: A clean, green extraction technology. Trends Ana. Chem. 2015;(71):100–09. https://doi.org/10.1016/j.trac.2015.04.013
11. Al-Ogaili N. The evaluation of total flavonoids, total phenolic content and biological activity of Iraqi *Lipedium sativum* L. crude extract obtained by optimized ultrasound-assisted extraction conditions. PST. 2024;11(2):214–20. https://doi.org/10.14719/pst.2975
12. Bargah RK. Preliminary test of phytochemical screening of crude ethanolic and aqueous extract of *Moringa pterygosperma* Gaertn. J Pharmacogn Phytochem. 2015;4(1):7–9.
13. Patel A. Estimation of flavonoid, polyphenolic content and *in vitro* antioxidant capacity of leaves of *Tephrosia purpurea* Linn. (Leguminosae). Int J Pharma Sci and Res. 2010;1(1):66–77.
14. Shi P, Du W, Wang Y, Teng X, Chen X, Ye L. Total phenolic, flavonoid content and antioxidant activity of bulbs, leaves and flowers made from *Eleutherine bulbosa* (Mill.) Urb. Int J Food Sci Nutr. 2019;7(1):148–54. https://doi.org/10.1002/fsn3.834
15. Fumarola L, Spinelli R, Brandonisio O. *In vitro* assays for evaluation of drug activity against Leishmania spp. Microbiol Res. 2004;(155):224–30. https://doi.org/10.1016/j.resmic.2004.01.001
16. Diwathe MC, Mazumdar B, Jayapal A. Ultrasound-assisted Extraction and Quantification of Antioxidant Activity and Phenolic Content of *Cordia dichotoma* Leaf Extracts. AIJR Abstracts. 2024:25.
17. Qu C, Yu S, Luo L, Zhao Y, Huang Y. Optimization of ultrasonic extraction of polysaccharides from *Ziziphus jujuba* Mill. by response surface methodology. Chem Cent J. 2013;(7):160. https://doi.org/10.1186/1752-153X-7-160
18. Ghafoor K, Choi YH, Jeon JY, Jo IH. Optimization of ultrasound-assisted extraction of phenolic compounds, antioxidants and anthocyanins from grape (*Vitis vinifera*) seeds. J Agric Food Chem. 2009;57(11):4988–94. https://doi.org/10.1021/jf9001439
19. Hosseini SS, Khodaiyan F, Kazemi M, Najari Z. Optimization and characterization of pectin extracted from sour orange peel by ultrasound-assisted method. Int J Biol Macromol. 2019;(125):621–29. https://doi.org/10.1016/j.ijbiomac.2018.12.096
20. Kumara K, Srivastava S, Sharanagatb VS. Ultrasound assisted extraction (UAE) of bioactive compounds from fruit and vegetable processing by-products: A review. Ultrason Sonochem. 2021; (70):105325. https://doi.org/10.1016/j.ultsonch.2020.105325
21. Ratananikom K, Premprayoon K. Ultrasonic-assisted extraction of phenolic compounds, flavonoids and antioxidants from Dill (*Anethum graveolens* L.). Scientifica. 2022;(1):1–6. https://doi.org/10.1155/2022/3848261
22. Garcia-Castello EM, Rodriguez-Lopez AD, Mayor L, Ballesteros R, Conidi C, Cassano A. Optimization of conventional and ultrasound -assisted extraction of flavonoids from grapefruit (*Citrus paradisi* L.) solid wastes. LWT-Food Sci Technol. 2015;64(2):1114–22. https://doi.org/10.1016/j.lwt.2015.07.024
23. Anahí J, Enríquez B, Reyes-Ventura E, Socorro J, Rodríguez V, Moreno-Vilet L. Effect of ultrasound-assisted extraction

parameters on total polyphenols and their antioxidant activity from mango residues (*Mangifera indica* L. var. Manililla). Sep. 2021;8:94. https://doi.org/10.3390/separations8070094

24. González-Centeno MR, Knoerzer K, Sabarez H, Simal S, Rosselló C, Femenia A. Effect of acoustic frequency and power density on the aqueous ultrasonic-assisted extraction of grape pomace (*Vitis vinifera* L.) a response surface approach. Ultrason Sonochem. 2014;21(6):2176–84. https://doi.org/10.1016/j.ultsonch.2014.01.021

25. Liao J, Xue H, Li J, Peng L. Effects of ultrasound frequency and process variables of modified ultrasound-assisted extraction on the extraction of anthocyanin from strawberry fruit. Food Sci Technol (Campinas). 2022;42(4):e20922. https://doi.org/10.1590/fst.20922

26. Mason TJ, A.J. Cobley AJ, Graves JE, Morgan D. New evidence for the inverse dependence of mechanical and chemical effects on the frequency of ultrasound. Ultrason Sonochem. 2011;18(1):226–30. https://doi.org/10.1016/j.ultsonch.2010.05.008

27. Najib A, Ahmad AR, Handayani V. ELISA Test on *Cordia myxa* L. leaf extract for α-glucosidase inhibitor. Pharmacogn J. 2019;11(2):358–61. https://doi.org/10.5530/pj.2019.11.54

28. Sahu M, Saxena K, Sahoo J, Swain SR. Phytochemical investigation, characterization of ethanolic extract of *Cordia myxa* leaves using LCMS/MS and their in-silico studies for wound healing activity. IJSTM. 2023;(12)12:11–28.

29. Shamkhy BM, Abbas YK. The biological activity of alcoholic extracts of *Cordia myxa*plant against Klebsiella isolated from infected patients. IJFMT. 2021;15(4):1435–39. https://doi.org/10.37506/ijfmt.v15i4.16910

30. Malik A, Ahmad AR. Determination of phenolic and flavonoid contents of ethanolic extract of Kanunang leaves (*Cordia myxa* L.). Int J Pharmtech Res. 2014-2015;7(2):243–46.

31. Debiasia BW, Raiserb AL, Douradoc SHA, Torresb M, Andrighettib CR, Bonacorsib C, et al. Phytochemical screening of *Cordia glabrata* (MART.) A.DC. extracts and their potential antioxidant, photoprotective, antimicrobial and antiviral activities. Braz J Biol. 2023;83:e248083. https://doi.org/10.1590/1519-6984.248083

32. Jasiem TM, ALmugdadi SFH, Aljubory IS, Latef QN. Phytochemical study and antibacterial activity of crude alkaloids and mucilage of *Cordia myxa* in Iraq. Int J Pharm Sci Rev Res. 2016;39(1),45:232–36.

33. Al-Maliki ADM, Aboud MN, Hameed MF. Estimation and evaluation of medicinal efficacy of maximum inhibitory concentration of alkaloids extracted from Iraqi *Cordia myxa* leaves against some pathogenic bacteria. J Phys Conf Ser. 2021;(2063):012020. https://doi.org/10.1088/1742-6596/2063/1/012020

34. Shwaish T, Al-Imarah FJM. Chemical composition of *Cordia Myxa* fruit: Phytochemical screening and identification of some bioactive compounds. Int J Adv Res. 2017;5(9):1255–60. https://doi.org/10.21474/IJAR01/5447

35. Akhtar N, Mirza B. Phytochemical analysis and comprehensive evaluation of antimicrobial and antioxidant properties of 61 medicinal plant species. Arab J Chem. 2018;11(8):1223–35. https://doi.org/10.1016/j.arabjc.2015.01.013

36. Tiwari U, Cummins E. Factors influencing levels of phytochemicals in selected fruit and vegetables during pre- and post-harvest food processing operations. Int Food Res. 2013;50(2):497–506. https://doi.org/10.1016/j.foodres.2011.09.007

37. Afzal M, Obuekwe C, Khan AR, Barakat H. Influence of *Cordia myxa* on chemically induced oxidative stress. FNS. 2009;39(1):6–15. https://doi.org/10.1108/00346650910930761

38. Aberoumand A, Deokule SS. Comparison of phenolic compounds of some edible plants of Iran and India. Pak J Nutr. 2008;7(4):582–85. https://doi.org/10.3923/pjn.2008.582.585

39. Al-Musawi MH, Ibrahim KM, Albukhaty S. *In vitro* study of antioxidant, antibacterial and cytotoxicity properties of *Cordia myxa* fruit extract. Iran J Microbiol. 2022;14(1):97–103. https://doi.org/10.18502/ijm.v14i1.8810

40. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods. 1983;(65):55–63. https://doi.org/10.1016/0022-1759(83)90303-4

41. Saki J, Khademvatan S, Pazyar N, Eskandari A, Tamoradi A, Nazari P. In vitro activity of *Cordia myxa*mucilage extract against Leishmania major and *L. infantum*promastigotes. Jundishapur J Microbiol. 2015;8(3):e19640. https://doi.org/10.5812/jjm.19640

42. Toutain PL, Ferran A, Bousquet-Mélou A. Species differences in pharmacokinetics and pharmacodynamics. Handb Exp Pharmacol. 2010:(199):19–48. https://doi.org/10.1007/978-3-642-10324-7_2

43. Aberoumand A. Natural compounds with antioxidant properties in selected plant foods. Int J Agric Food Sci. 2011;1(2):27–29. https://doi.org/10.5812/jjm.19640

44. Moreno D, Plano D, Baquedano Y, Jimenez-Ruiz A, Palop JA, Sanmartin C. Antileishmanial activity of imidothiocarbamates and imidoselenocarbamates. Parasitol Res. 2011;108(1):233–3 9. https://doi.org/10.1007/s00436-010-2073-x

45. Antwi CA, Amisigo CM, Adjimani JP, Gwira TM. *In vitro* activity and mode of action of phenolic compounds on *Leishmania donova*ni. PLOS Negl Trop Dis. 2019;13(2):e0007206. https://doi.org/10.1371/journal.pntd.0007206

46. Kostyuk V, Potapovich A, Strigunova E, Kostyuk T, Afanas'ev I. Experimental evidence that flavonoid metal complexes may act as mimics of superoxide dismutase. Arch Biochem Biophys. 2004;(428):204–08. https://doi.org/10.1016/j.abb.2004.06.008

47. Silva-Silva JV, Moragas-Tellis CJ, Chagas MSS, Ramos de Souza PV, Freitas de Souza CS, Hardoim DJ, Taniwaki NN, et.al. Antileishmanial activity of flavones-rich fraction from *Arrabidaea chica Verlot* (Bignoniaceae). Front Pharmacol. 2021;(12):703985. https://doi.org/10.3389/fphar.2021.703985

48. Chauhan K, Kaur G, Kaur S. Activity of rutin, a potent flavonoid against SSG-sensitive and -resistant *Leishmania donovani* parasites in experimental leishmaniasis. Int Immunopharmacol. 2018;64):372–85. https://doi.org/10.1016/j.intimp.2018.09.026

49. Perez-Victoria JM, Chiquero MJ, Conseil G, Dayan G, Di Pietro A, Barron D, Castanys S, Gamarro F. Correlation between the affinity of flavonoids binding to the cytosolic site of *Leishmania tropica* multidrug transporter and their efficiency to revert parasite resistance to daunomycin. Biochemistry.1999;38(6):1736–43. https://doi.org/10.1021/bi982455v

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